or almost so; wash with as little 95 per cent ethyl alcohol as possible (3 to 4 cc.), and follow with a wash of 1 or 2 cc. of ether; remove the solid from the filter paper and dry on a watch-glass at 90° C. The solid obtained is the amido-H-acid of the coupling product used. The same procedure applies to dyes from H-acid; if the dye-amount taken be less than one gram the proportion of reagents used should be altered to correspond.

The three identification tests used were as follows:

I—Boil a fraction of a milligram of the free acid with a few drops of 40 per cent caustic soda solution, dilute with 1 cc. water and note color produced; boiling the diluted solution may at times be necessary.

II—Place a fraction of a milligram of the free acid into 2 or 3 drops conc. H_2SO_4 (sp. gr. 1.84) and warm until it *begins* to turn violet; add one or two drops 0.5 per cent sodium nitrite solution; cool; dilute with 0.5 cc. water; cool (if a brown opalescence or turbidity appears here start all over again and do not heat quite so high); neutralize largely with 40 per cent caustic soda, finish neutralizing and make alkaline with slight excess saturated carbonate of soda solution; note color change.

III—Dissolve a fraction of a milligram of the free acid in one drop of saturated carbonate of soda solution, using heat if need be; add one drop concentrated hydrochloric acid, dilute with water to 0.5 cc., heat to boiling and cool; place a drop of this on good white filter paper; add right on top of it a drop or two of good hydrogen peroxide solution; when the color-development has reached its maximum, add a drop of 40 per cent caustic soda solution, preferably right on top of the other drops. Note color changes.

The behavior of these acids is tabulated as follows:

Test	2-Acid	7-Acid	2,7-Acid
I..................	Yellow or brown	Green	Violet going to blue
II..................	Blue-red	Blue-red	Yellow or brown
III H_2O_2..........	Brown or yellow	Violet to red	Blue
NaOH............	Red	White or cream colored	Red to violet

By suitably combining these tests it is possible to detect all three of these acids in mixtures of 90 : 5 : 5 of any combination; with care, 200 mg. of H-acid-dye will answer to carry out this separation and all three of these tests. The colors produced by Test III can be photographed, *e. g.*, on a Lumière plate and the colors so preserved for reference; they are not stable, as such.

Condensation with phenanthrene-quinone is not satisfactory, calling for much time and care, and frequently failing where it should succeed; moreover, the amounts needed are very much larger than in the tests described; 2-amido-H-acid gives such a condensation product which is yellow, soluble in carbonate of soda and is precipitated therefrom by common salt and dissolves in concentrated sulfuric acid with a cornflower-blue color; the 2,7-diamido-H-acid does not yield such a condensation product under any practicable condition nor does the 7-amido-H-acid.

Acknowledgments are herewith made to Messrs. Read Holliday and Sons, of Huddersfield, England, for permission to publish this note.

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INDIGOTIN CONTENT OF SOME JAPANESE INDIGOES[1]

By Shinjiro Sato

Received April 8, 1915

The analyses given below were made on commercial samples from the Province of Awa, where nearly all the Japanese indigo is produced. The remarkable features of the results are the low indigotin content and high ash. These are not surprising, however, when considered in relation to the methods of production, which have been modified but little since the introduction of indigo culture into Japan over two hundred years ago.[2]

In the native process the dried leaves of the *Polygonum tinctoria* are heaped on dirt floors, then thoroughly moistened with water and allowed to ferment. The piles are turned over from time to time, the whole process requiring about three months. The fermented mass, called "sukumo," is pounded up in large wooden mortars with mallets, then mixed with water and left to harden. The resulting cake is pressed and cut into cubes, called "tamas" ("Tama," Japanese word for "gem") in which form it is put on the market.

The above method requires much time and labor and yields a product of low indigotin content, since there is no extraction to separate the dye from the fiber of the plant. An extraction method applicable to Japanese conditions has been perfected by Dr. N. Nagai.[3] Although the method requires less time and labor than the older process and produces a superior indigo, it has not yet been generally adopted.

METHOD OF ANALYSIS

To test out the methods of analysis pure indigotin was first prepared, starting with Indigo S Pdr. furnished by the Badische Co. This was purified according to the method of Mönlau and Zimmermann,[4] by dissolving in 100 parts glacial acetic acid and 4 parts sulfuric acid, then hydrolyzing by diluting with water. The precipitated indigotin was washed, dried at 110°, and nitrogen determined by the Kjeldahl method.

	Found	Theoretical	
N	10.62 per cent	10.69 per cent	Indigotin 99.35 per cent

The method adopted for extracting and estimating indigotin is a modification of Rawson's procedure[5] in which he extracted indigotin by sulfonation and titrated with $N/50$ permanganate. The original method was modified by its author[6] and later by Bergtheil and Briggs,[7] whose procedure was adhered to exactly in the following analyses. The above methods for preparation of pure indigotin and for analysis of commercial indigos have been alternately criticized and defended.[8]

RESULTS OF ANALYSIS

Ash and indigotin are calculated on weight of dry sample. In all cases duplicates represent separate extractions.

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[1] Partial abstract of a thesis presented to the Faculty of the State College of Washington in part fulfillment of the requirements for the B.S. degree.

[2] History of Awa Indigo. (Published in Japanese.)

[3] Nagai, Lectures on Awa Indigo. (Published in Japanese.)

[4] *Zeits. Farb. Text. Chem.*, **10** (1903), 189.

[5] *J. Soc. Dyers and Col.*, **1** (1885), 74.

[6] *J. Soc. Chem. Ind.*, **35** (1906), 730.

[7] *Ibid.*, **18** (1899), 251; **25** (1906), 734.

[8] *Ibid.*, **26** (1907), 4, 182, 1172, 1174.

Sample	Per cent moisture	Per cent ash	Per cent indigotin
Purified synthetic indigo	....	....	99.71
	....		100.59
Awa indigo No. 1	9.71	27.75	6.78
	9.41	27.41	6.86
Awa indigo No. 2	9.18	27.28	5.05
	9.30	27.17	5.15
Awa indigo No. 3	10.08	30.56	4.23
	10.10	30.47	4.32
Awa indigo No. 4	12.44	32.39	1.94
	12.44	32.48	2.02
Awa indigo No. 5	9.24	33.89	1.26
	9.21	33.77	1.34
Awa indigo No. 6	11.56	34.50	2.46
	11.56	34.55	2.47
Awa indigo No. 7	9.92	34.34	3.17
	9.95	34.32	3.23
Awa indigo No. 8	9.46	35.26	3.59
	9.38	35.08	3.72
Indigo by Nagai process	3.18	33.25	31.45
	3.28	33.25	31.53

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EXPERIMENTAL DATA COMPARING THE DELICACY OF DIFFERENT TESTS FOR HYDROGEN PEROXIDE IN MILK

By Irwin T. Darlington

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From references on the detection of hydrogen peroxide, the reagents listed in Table I were found to be in most general use and appear to give the most delicate tests for the detection of hydrogen peroxide in milk.

A stock solution of hydrogen peroxide was made from a commercial sample containing 3.2 per cent of hydrogen peroxide by analysis with potassium permanganate. This stock solution was made so that each cc. contained 0.01 g. of hydrogen peroxide.

Table I

Reagent	Solution	Color in presence of H_2O_2
Starch	Dilute (water) + KI	Bluish green
Paraphenylene diamine	2 per cent (water)	Blue
Benzidine	4 per cent (alcoholic) + 10 cc. milk (+ 2 or 3 drops acetic acid)	Blue
Titanic acid	2 per cent solution titanium hydrate in dilute H_2SO_4	Yellow
Vanadic acid	2 per cent in dilute H_2SO_4	Faint orange-red

The milk to which the hydrogen peroxide was added was received from the city inspectors and was proven free from hydrogen peroxide by each of the above reagents.

Hydrogen peroxide was added to each 100 cc. of milk in separate bottles to the amounts indicated in Table II, and tests made on 10 cc. of each sample with each of the above reagents. Tests were made immediately after the samples were made up and again after standing 18 hours in a warm room.

Table II

Grams per 100 cc.	Results of tests made immediately after samples were made up																	After standing 18 hrs. in a warm room		
	15.0	9.0	3.0	1.5	0.75	0.6	0.3	0.15	0.03	0.015	0.006	0.003	0.006	0.003	0.00105	0.00075	0.00037	15.0	9.0	3.0
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—
Paraphenylene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—
Benzidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—
Titanic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—
Vanadic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—

It will be seen from Table II that paraphenylene diamine and benzidine proved to give the most delicate tests for hydrogen peroxide in raw milk. The limits of these two reagents proved to be 0.00075 g. of hydrogen peroxide in 100 cc. of raw milk. No experiments were made with milk that had been previously heated to destroy bacterial action.

Practically all the hydrogen peroxide was reduced after standing 18 hours in a warm room. This confirmed previous results obtained in this laboratory when it was found that after 18 hours' standing at room temperature no reaction could be obtained from the addition of 1 g. of magnesium peroxide to 100 cc. of raw milk. Positive tests were given only when the milk contained 9 per cent and 15 per cent of hydrogen peroxide.

The samples containing 9 per cent and 15 per cent of hydrogen peroxide failed to give a positive test with any of the above reagents, except paraphenylene diamine, which gave a delicate positive test with 15 per cent of hydrogen peroxide after standing in a warm room for 24 hours. Tests on distillates of milk treated with peroxide were negative.

A few tests were made with magnesium dioxide added as a preservative. The experiments made with this material were insufficient to afford any positive conclusions, but it appears that magnesium dioxide does not lend itself to as delicate tests as does hydrogen peroxide.

From the foregoing it is seen that paraphenylene diamine is the test of the reagents tested for the detection of small quantities of hydrogen peroxide in raw milk, and that it is probable that peroxide in amounts that would be added to milk as a preservative cannot be detected after 18 hours by these reagents.

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THE OFFICIAL METHOD FOR DETERMINING CRUDE FIBER AS APPLIED TO COTTONSEED MEAL

By C. K. Francis

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The well known Weende method[1] for determining crude fiber has been subjected to criticism by many chemists. The chief trouble seems to be with the filtering materials, linen, asbestos, or glasswool, which vary in their physical characteristics and, necessarily, in filtering efficiency. In a number of laboratories it has been the practice to use linen cloth for filtering. The cloth has been used in the same manner as filter paper, with and without suction, in plain and in ribbed funnels. It has been difficult to duplicate linen of a selected pick, so that the fineness of the filter has been liable to vary from time to time. However, the method seemed to be satisfactory when working with such substances as corn, but when one endeavored to filter the alkaline mixture of certain seeds like flaxseed and cottonseed it became necessary to devise some scheme to hasten the process.

A novel arrangement for filtering the mixture through cloth by upward suction has been suggested by Pickel.[2] The cloth stretched over a funnel or thistle-tube furnishes a flat surface and the liquid passes rapidly,

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[1] "Official Methods of Analysis," U. S. Bureau of Chemistry, *Bull.* **107** (Rev.), 65.

[2] This Journal, **2** (1910), 280; see MacNider, *Ibid.*, 281.